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EFFECT OF HIGH AND LOW ERUCIC ACID RAPESEED OIL ON THE LIVER OF THE CHICK

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

AND RESEARCH IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Effect of high and low erucic acid rapeseed oil on the liver of the chick" submitted by Deborah Lynn MacLellan, in partial fulfillment of the requirements for the degree of Master of Science in Nutrition.

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ABSTRACT

Duplicate experiments were conducted to determine the effect of incorporation of rapeseed oil into the diet of the chick on liver size, hydroxyproline content of liver protein, lipid content of the liver and the fatty acid composition of neutral liver lipid and liver phospholipid. For comparative purposes the fatty acid composition of carcass lipid was also determined. The diets fed contained high erucic acid rapeseed oil (HER), low erucic acid rapeseed oil (LER), or sunflowerseed oil (SFO) and were formulated by substitution of 20 parts oil isoenergetically for glucose. All chicks were fed the experimental diets from 4 to 28 days of age.

When fed ad libitum, it was found that chicks fed diets containing HER consumed fewer calories and grew at a slower rate than LER or SFO fed chicks. Results also showed that growth and caloric consumption of chicks fed diets containing LER was intermediate between and significantly different from that of chicks fed either the HER or SFO containing diets.

Chicks fed diets containing HER had significantly heavier livers than chicks fed LER containing diets, which in turn, were found to have significantly heavier livers than chicks fed diets containing SFO. Results of liver lipid determinations showed that chicks fed diets containing HER had a significantly higher percentage of

lipid in their livers than did the SFO fed controls. The level of lipid in livers of chicks fed LER was intermediate between but not significantly different from that of chicks fed diets containing SFO and HER. Level of phospholipid in liver lipid was similar irrespective of whether diets contained HER, LER or SFO. No difference was observed in the percentage of hydroxyproline in liver protein of chicks fed HER, LER and SFO, thus indicating that collagen, the chief constituent of connective tissue, was not increased.

Marked differences in fatty acid composition of liver neutral lipids and phospholipids were observed when diets containing HER, LER and SFO were fed. Both the neutral lipids and phospholipids in liver of chicks fed diets containing HER and LER were characterized by having lower levels of stearic, linoleic and arachidonic acids and higher levels of oleic acid than when diets containing SFO were fed. The liver neutral lipids and phospholipids of chicks fed diets containing HER differed from those of chicks fed diets containing LER in that they contained erucic acid and also had higher levels of eicosenoic and lower levels of linoleic and arachidonic acids. Whether changes in fatty acid composition of phospholipids contribute to structural - functional changes in the liver and to the observed increase in liver size when diets containing HER and LER rather than SFO were fed is unknown.

Liver neutral lipids of chicks were found to be more saturated than carcass lipid and when HER was fed liver neutral lipids were found to contain slightly but significantly lower levels of erucic and eicosenoic acids. than carcass lipid. Differences in rates of oxidation of palmitic and erucic acid by extrahepatic tissue may contribute to these differences and thus do not necessarily negate the hypothesis that in the chick the liver helps to protect other tissues from excessive exposure to erucic and eicosenoic acids.

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INTRODUCTION

Incorporation of high erucic acid rapeseed oil in the diet of both chicks and rats has been shown to decrease growth, to decrease feed consumption and to decrease efficiency of utilization of energy. Unlike the rat, however, incorporation of high erucic acid rapeseed oil in the diet of the chick results in increased heart size, but does not cause cardiac lipidosis or accumulation of erucic acid in heart lipid.

Fat metabolism in chicks differs from that in rats in several respects. It is now established that in chicks triglycerides are conveyed from the intestinal tract to the liver via the portal vein while in albino rats absorption of triglycerides is via the lymphatic system. In addition, studies have shown that while the liver is the major site of fat synthesis in chicks, in rats fat synthesis occurs not only in the liver but also in the adipose tissue. Thus, the possibility exists that the liver of the chicken may alter the fatty acid composition of the ingested rapeseed oil sufficiently to prevent cardiac lipidosis and myocardial damage.

Although a great deal of information has accumulated on the effect of both high and low erucic acid rapeseed oils on the liver of the rat, very little work has been reported using the chicken as the experimental

animal. Thus the following studies were conducted to determine the effect of feeding diets containing high and low erucic acid rapeseed oil on the liver of chicks.

LITERATURE REVIEW

Effect of rapeseed oil on growth

It has been well established that animals fed diets containing high erucic acid rapeseed oil grow less than animals fed diets containing such other oils as corn oil, soybean oil or sunflowerseed oil. In studies to determine the nutritive value of rapeseed oil for the chick, Clement and Renner (1977) showed that high erucic acid rapeseed oil (HER) in excess of 10% by weight in the diet resulted in reduced feed intake and reduced growth. Sheppard et al. (1971) have also shown that chicks fed diets containing 16% rapeseed oil (32% erucic acid) grew slower and utilized their feed less efficiently than chicks fed corn oil diets.

Studies have also been conducted to determine the nutritive value for the chick of new varieties of rapeseed oil containing lower levels of erucic acid. Vogtmann et al. (1973) studied the growth and feed efficiency of chicks fed diets containing 5, 10 and 15% of various rapeseed oils, soybean oil and lard. The three experimental rapeseed oils which he used contained 21.4, 2.9 and 2.8% erucic acid. In agreement with Sell and Hodgson (1962) and Renner (1967), Vogtmann et al. (1973) showed that rapeseed oil, irrespective of erucic acid content, promoted growth equal to either soybean oil or lard when fed at the 5 or 10% level. At the 15% level, chicks fed the regular rapeseed oil containing 21.4%

erucic acid grew significantly slower than chicks fed diets containing 15% lard or 15% soybean oil, but utilized their feed just as efficiently. The growth of chicks fed the low erucic acid rapeseed oils at the 15% level was variable. One sample resulted in growth depression while the other resulted in growth similar to that of the soybean oil fed controls. The authors offered no explanation for this variability.

More extensive studies on the nutritive value of rapeseed oil have been carried out using the rat as the experimental animal. As was observed in the chick, these studies showed that incorporation of high erucic acid rapeseed oil in excess of 10% by weight of the diet reduced growth and feed consumption (Beare et al., 1957; Thomasson and Boldingh, 1955; Beare et al., 1959b; Hornstra, 1972 and Craig et al., 1963a). Beare et al. (1959a) showed that when body weight gains were adjusted for feed consumption by covariance analysis, differences largely disappeared. These results indicated that decreased appetite could be a factor contributing to the decreased growth observed in animals fed rapeseed oil.

One of the growth depressing factors in rapeseed oil was shown by Thomasson and Boldingh (1955) to be erucic acid. Beare et al. (1959a) confirmed these results when they found that substitution of increasing levels of ethyl erucate for corn in the diet of weanling rats caused a progressive decrease in growth. Additional confirmation that erucic acid contributes to the growth

depression is obtained from the finding that growth of rats and chicks fed diets containing LER is greater than when diets containing HER are fed. (Craig and Beare, 1968; Rocquelin and Cluzan, 1968).

The unfavorable ratio of saturated fatty acids to unsaturated fatty acids has also been postulated to be a factor in rapeseed oil which may contribute to its growth depressing properties. Beare et al.. (1963) showed that increasing the palmitic acid content of rapeseed oil from 3 to 24% by the addition of palm oil significantly increased growth of rats fed oils containing 20% erucic acid and approximately 16% linoleic acid. However, in a subsequent experiment, Beare-Rogers et al. (1972) showed that increasing the level of palmitic acid from 3.5% to 19.1% in a mixture containing 32% erucic acid and 18.7% linoleic acid did not affect food intake or growth.

Effect of rapeseed oil on energy utilization

Recent studies (Clement and Renner, 1977) have shown that the isocaloric substitution of 20 parts of either high or low erucic acid rapeseed oil for sunflower-seed oil in the chicks' diet under conditions of equalized nutrient intake resulted in decreased fat deposition and decreased efficiency of energy utilization when energy gained as a percent of energy consumed was used as the criterion of efficiency. That the decrease in efficiency of energy utilization observed in vivo was apparently

related to the efficiency of mitochondrial oxidative phosphorylation was shown in subsequent experiments (Renner et al., 1979) which showed a reduction in ADP/O ratios, reduced rates of ATP synthesis and changes in the fatty acid composition of cardiac mitochondrial membranes when either high or low erucic acid rapeseed oil was substituted for sunflowerseed oil in chick diets.

In rats, dietary substitution of HER for SFO has been associated with decreased energetic efficiency measured as decreased body weight gain per unit of digestible energy consumed and increased rate of oxygen consumption (Hornstra, 1972). These metabolic changes have been associated with uncoupling of oxidative phosphorylation (Hornstra, 1972). Impaired energy utilization has been observed for the rat in some (Houtsmuller et al., 1970; Hornstra, 1972) but not all (Dow-Walsh et al., 1975) short term studies of oxidative phosphorylation by cardiac mitochondria isolated from rats fed diets containing HER. Prolonged feeding of HER and LER has been shown to alter the efficiency of utilization of a variety of mitochondrial substrates in vitro (Hsu and Kummerow, 1977; Clandinin, 1978). Other evidence suggesting that dietary rapeseed oils induced changes in mitochondrial function arose from studies to determine the mechanism of triglyceride accumulation in cardiac tissue of rats fed diets containing HER. In this regard, fat accumulation has been attributed to the following factors: slower rate of oxidation of

erucic acid due to reduced activity of enzymes of fatty acid activation (Blond et al., 1975; Cheng and Pande, 1975); reduced activity of enzymes of fatty acid oxidation (Swarttouw, 1974); inhibition of mitochondrial oxidation of fatty acids by a mitochondrial metabolite of erucic acid (Christopherson and Bremer, 1972); difficulties in the oxidation of erucic acid involving its carnitine dependent transport to the sites of B-oxidation (Bulhak-Jachymczyh and Hubner-Wozniak, 1974); impaired ATP utilization via ATP translocase creatine phosphokinase system (Blomstrand and Svensson, 1975; Clandinin, 1978), and dietary fat induced changes in the fatty acid composition of phospholipids integral to the function of mitochondrial enzymes (Clandinin, 1978; Blomstrand and Svensson, 1974; Dewailly et al., 1977).

Effect of rapeseed oil on liver size

Chickens

Little information is available on the effect on the liver of feeding high and low erucic acid rapeseed oil to chickens. Sheppard et al. (1971) fed diets containing 16% by weight rapeseed, crambe or corn oil to chicks for three weeks. They found that there was no significant difference between the average liver weights of chicks fed the crambe and rapeseed oil diets, but the average liver weight of chicks fed the corn oil diet was significantly lower than those of chicks fed the other two diets.

Vogtmann et al. (1974) found that both the kind and amount of fat included in the diet had a signifcant effect on the liver weight of chicks. When organ to body weight ratios were analyzed statistically, it was found that high erucic acid rapeseed oil, but not low erucic acid rapeseed oil increased the liver to body weight ratio significantly. In a subsequent experiment, Vogtmann et al. (1978) reported that significantly larger livers were found in chicks fed rations containing 15% rapeseed oil (22% erucic acid) from 1 to 10 days of age than in chicks fed rations containing 15% soybean oil. Salmon (1969) fed turkey poults a diet containing degummed rapeseed oil, beef fat, or a series of blends of the two fats at a level of 10% of the diet. Measurement of the liver weights at six weeks revealed a significant enlargement when diets contained either 10% rapeseed or 7.5% rapeseed oil plus 2.5% beef fat. The differences were highly significant when expressed as a percent of body weight. Ratanasethkul et al. (1976) fed rations containing 25% of either regular rapeseed oil (36% erucic acid), Oro rapeseed oil (1.9% erucic acid), soybean oil or a mixture of lard and corn oil to chickens, ducks and turkeys. They found that the average relative weight of the livers of all species fed the regular rapeseed oil ration was greater than those of the same species fed the other rations with the difference being most marked for ducks. Results with chickens showed that the increase in liver to body weight when diets

containing regular rapeseed oil was fed was significant at 28 and 52 days of age. They observed no significant differences in relative liver weight when chickens were fed diets containing soybean oil, Oro rapeseed oil or a mixture of lard and corn oil.

Rats

Much more information is available on the effect on the liver of feeding rapeseed oil to rats. In 1951, Carroll fed Sprague-Dawley rats a diet containing 25% rapeseed oil (HER) for four weeks. He found some increase in the weight of the liver and a marked increase in its content of esterified cholesterol. Beare et al. (1957) fed Wistar rats diets containing corn oil, Canadian produced rapeseed oil (40% erucic acid) or mixtures of these two oils containing 5, 10, 20, 40 and 80% rapeseed oil at levels of 10 and 20% by weight of the diet. Neither the level of dietary fat, nor the proportion of rapeseed oil in the fat mixture was found to affect either absolute or relative liver weight. Subsequently, Beare et al. (1959b) compared the utilization of rapeseed oil and corn oil by the rat. Wistar rats were fed, ad libitum, a diet consisting of rapeseed oil (HER) or corn oil for one to five weeks. Absolute liver weights were significantly greater for the corn oil fed animals at three, four, and five weeks time, however, adjustment of both groups to the same body weight indicated that the rapeseed oil group had

significantly heavier livers. The following year, Beare et al. (1960) repeated this experiment using two strains of rats. Both Wistar and Sprague-Dawley rats were fed, ad libitum, either 20% rapeseed oil or corn oil in a basal diet of ground fox cubes for six weeks. Absolute liver weights were found to be greater in Sprague-Dawley rats, but in neither strain was absolute liver weight affected by the dietary oil. However, when liver weights were adjusted for body weights by a covariance analysis, the livers of rats fed rapeseed oil were found to be significantly heavier, and the strain effect was found to be negligible. In a subsequent experiment (Beare et al., 1963), rats were fed diets containing Polish rapeseed oil, Swedish rapeseed oil supplemented with palm oil or a mixture of lard and olive oil. It was found that neither absolute liver weight nor liver to body weight ratio varied significantly between treatments. Abdellatif et al. (1973) showed that animals fed diets containing rapeseed oil in amounts to supply 25% or more of the energy in the diet for 32 weeks had significantly greater liver to body weight ratios than when diets containing sunflowerseed oil were fed.

Effect of rapeseed oil on level of liver lipid

Chickens

The effect of feeding diets containing rapeseed oil on the accumulation of fat in the liver has also been

studied in both chicks and rats, however, results have been conflicting. Sheppard et al. (1971) fed day old chicks diets containing either no added oil or 16% by weight corn oil, crambe oil, rapeseed oil (HER), ad libitum for three weeks. They found that the chicks deposited similar amounts of fat in the liver, regardless of the oil fed. Vogtmann et al. (1974) fed diets containing 5, 10 and 15% of rapeseed oils of high and low erucic acid content to four week old chickens for 28 days and reported that the total lipid content of the liver was not influenced by the kind of oil or fat included in the ration. Subsequently, Vogtmann et al. (1978) showed that the livers of chicks fed diets containing 15% regular rapeseed oil had similar levels of lipid as chicks fed diets containing 15% soybean oil. They observed that irrespective of the fat fed, total lipid in liver tissue declined from 1 to 10 days of age while the percentage of lipid in the heart tissue gradually increased from 1 to 10 days of age.

Rats

Rocquelin et al. (1970) reported that livers of male rats fed a diet containing 15% by weight of either rapeseed oil (HER), canbra oil, or peanut oil for 2 months did not differ in fat content. In a subsequent experiment, Rocquelin (1973), fed male Wistar rats diets containing 15% by weight of either refined, crude or interesterified rapeseed oil (HER). He reported that the level of liver

lipid in rats fed diets containing the refined oil appeared to be normal and similar in amount to level of liver lipid in rats fed peanut oil. He observed that the level of liver lipid was slightly but significantly higher when interesterified oil was fed and lower when raw oil was fed in comparison to when the diet containing refined oil was fed. Beare-Rogers et al. (1971) also reported that the fat content of the liver did not vary when rats were fed diets containing either a lard:corn oil mixture, liquid rapeseed oil, partially hydrogenated rapeseed oil or partially hydrogenated herring oil; however, they did find that increasing amounts of fat were accumulated in the liver with increased time of feeding regardless of oil fed. Bulhak-Jachymczyh et al. (1974) also concluded that erucic acid consumption does not cause liver lipidosis. They fed male Wistar rats rapeseed oil containing 49.3% erucic acid for 4 hours prior to decapitation. An average of 400 milligrams of erucic acid was consumed by the rats fed the rapeseed oil. They found fatty infiltration induced by the dietary erucic acid only in the heart and skeletal muscles, and suggested that this was due to the dependence of these tissues on carnitine function alone for the oxidation of long chain fatty acids. They also suggested that the liver must be able to utilize an alternate pathway for metabolizing erucic acid.

In contrast, Abdellatif and Vles (1973) fed male rats diets in which blends of rapeseed oil and sunflowerseed

oil provided 40% of the energy and in which rapeseed oil (50% erucic acid) provided 0, 5, 10, 15, 20, 25 and 30% of the energy. They found a slight degree of fatty infiltration of the liver of rats fed the diets containing 25 and 30 percent of the energy as rapeseed oil (50% erucic acid) after 32 weeks. They found no pathological changes with Canbra oil (LER) when fed in amounts to provide 30, 50 or 60 percent of the calories. Kramer et al. (1973) fed male and female rats three types of rapeseed oils varying in their erucic acid content at 20 percent by weight of the diet for one and two weeks. They found that the fat content of livers of rats of both sexes fed Span rapeseed oil and HER to be statistically higher than that found in the livers of the control groups. Rats on diets containing Oro oil did not develop this fat accumulation. Kienle et al. (1976) also reported that level of total liver lipid from rats fed a diet containing rapeseed oil (46.6% erucic acid) in an amount to supply 25% of the energy was statistically higher than the total liver lipid from rats fed either a standard pellet diet or an olive oil containing diet.

Effect of rapeseed oil on fatty acid composition of liver lipid

Chickens

Studies on the effect of different oils on the fatty acid composition of liver lipid of chicks have been

reported by several authors. Vogtmann et al. (1974) in an experiment involving four week old chickens, found that the fatty acid composition of the feed fats altered the fatty acid pattern of the total liver lipids. Feeding rapeseed oil, containing 21.4% erucic acid, at levels of 5, 10 and 15% by weight in the diet led to a decrease in the concentrations of saturated fatty acids and an increase in concentration of monounsaturated fatty acids in liver lipids in comparison to chicks fed diets containing comparable levels of corn oil. The increase in concentration of monounsaturated fatty acids was mainly attributed to increases in the concentration of eicosenoic and erucic acids. When diets containing low erucic acid rapeseed oils were fed, similar but smaller changes in the composition of the tissue lipids of the chickens were observed. In a subsequent experiment, Vogtmann et al. (1978) fed chicks rations containing 15% rapeseed oil (HER) or soybean oil (SBO). They found that inclusion of rapeseed oil (HER) in the chicks' ration caused an increase in the concentration of monounsaturated fatty acids in the liver when compared to the livers of chicks fed SBO. It is interesting to note, however, than an appreciable decrease of oleic acid was noted in the liver tissue of the chickens from both ration treatments during the period of 1 to 10 days of age. The authors concluded that this demonstrated the importance of oleic acid as a source of energy for the chicken for the first few days after hatch. Leclerq (1972)

examined the fatty acid composition of the liver and carcass lipids of hens fed a 5% rapeseed oil diet containing 27.7% erucic acid. He found that more stearic acid and less linoleic and erucic acid were deposited in the liver as compared to the adipose tissue.

Rats

It is generally agreed that dietary fatty acids do alter the fatty acid composition of liver fat, however, Beare (1961) found that, in rats, the influence on carcass fat was greater than on liver lipids. She attributed this to the fact that a large proportion of liver fat is phospholipid, the fatty acid composition of which remains relatively constant. She fed a purified diet containing 20% by weight of corn oil, rapeseed oil (HER), hydrogenated herring oil, or a margarine containing some marine oil to male, weanling Wistar rats for six weeks. Lower levels of saturated fatty acids and higher levels of monounsaturated fatty acids were deposited in the tissues of rats fed the rapeseed oil diet as compared to the corn oil diet. Erucic acid was found in both the liver and carcass lipid although significantly higher levels were deposited in the carcass. In this and in a subsequent experiment, Beare et al. (1963) observed that when rapeseed oil was present in the diet, the level of arachidonic acid in the liver was less than when the diet contained no erucic acid. Beare et al. (1963) concluded that in the presence of erucic acid the conversion of linoleic

acid to arachidonic acid was decreased. Craig et al. (1963b) also showed that rats fed diets containing 20% HER deposited more erucic and eicosenoic acid in abdominal, carcass and cutaneous fat than in liver lipid. Separation of liver lipid into triglyceride and phospholipid fractions showed small amounts of erucic and eicosenoic acid in liver triglyceride while liver phospholipids contained eicosenoic but not erucic acid.

Kramer et al. (1973) fed fully refined rapeseed oils varying in their erucic acid content to male and female rats at 20% by weight in their diets to assess the effects of the low erucic acid varieties of rapeseed on fatty acid composition of liver lipid. They found that the ratio of saturated to unsaturated fatty acids in liver lipids was much lower in the rapeseed oil groups as compared to the control lard and corn oil fed groups. These lower ratios were the result of significantly lower saturated fatty acid concentrations and much elevated monounsaturated fatty acid concentrations in the livers of the rats fed the rapeseed oils. In a subsequent experiment Kramer (1973) investigated the effect of dietary rapeseed oils on the fatty acid composition of several major classes of liver lipid: neutral lipids, phosphatidylethanolamine (PE) and phosphatidycholine (PC). He fed male weanling rats various rapeseed oils which differed in their erucic acid concentration, at a level of 20% by weight for sixteen weeks. Erucic acid was

incorporated into the liver lipids of rats at levels of 0.1, 0.4 and 1.7% when diets containing Oro, Span and HER were fed. The concentration of erucic acid in the liver lipids was proportional to its concentration in the dietary rapeseed oils, but at much lower levels. In contrast to Craig et al. (1963b), Kramer (1973) found that neutral lipids and the phospholipids contained similar amounts of erucic acid. Although eicosenoic acid was found in the liver lipids of rats fed lard and corn oil, much higher concentrations were present in rats fed the high and low erucic acid rapeseed oils. The concentrations of eicosenoic acid appeared to be related to the dietary intake, although the contribution from B-oxidation of erucic acid and chain elongation of oleic acid cannot be eliminated.

A marked decrease in the concentration of palmitic acid was observed in all lipid classes of all groups fed rapeseed oil when compared to groups fed corn oil or lard. The concentration of linoleic acid in the neutral lipids was found to be related directly to its concentration in the diet, while the influence of dietary linoleic acid on phospholipid composition was found to be less marked. The relative proportions of stearic acid and arachidonic acid in the total lipids of rat livers was not statistically different between experiment groups; however, like Craig et al. (1963b), Kramer (1973) observed significantly higher levels of stearic and arachidonic acid in the liver

phospholipids as compared to the liver triglycerides.

Rogers (1977) investigated the effects of unlabelled and labeled erucic acid on the major neutral lipid and phospholipid components of a cell line of fetal rat liver epithelial cells. He reported that the amount of erucic acid taken up from the medium in 24 hours was comparable to that of rat myocardial cells, although with liver cells the initial rate of uptake was lower. He found that labelled erucic acid was incorporated to a greater extent into liver neutral lipids as compared to liver phospholipids.

Studies have also been conducted to compare the composition of lipid deposited in liver and heart when diets containing high erucic acid rapeseed oil were fed. Results showed that erucic and eicosenoic acids were deposited in liver but at lower levels than in the heart. (Kienle et al. 1976; Rocquelin, 1973; Walker, 1972).

Pathological effects of rapeseed oil on the liver

Birds

The pathological effects of dietary rapeseed oil on livers of several avian species has been investigated. Using Peking ducklings as the experimental animal, Abdellatif and Vles (1971) observed that incorporation of rapeseed oil (HER) in the diet in amounts to provide 30-60% of the energy resulted in cirrhotic changes in the liver. Further studies (Abdellatif et al., 1972) showed

that cirrhotic changes in the liver could be reduced when dietary energy was provided by a mixture of rapeseed oil (HER) and hardened palm oil rather than a mixture of rapeseed oil (HER) and soybean oil. Since cirrhotic changes in the liver of ducklings was not observed when the low erucic rapeseed oil, Oro, was fed it would appear that both high levels of erucic and low levels of saturated fatty acids in rapeseed oil (HER) contributed to pathological changes in the liver (Abdellatif and Vles, I973).

More recently Ratanasethkul et al. (1976) studied the pathological changes in livers of chickens, ducks and turkeys fed diets in which high and low erucic acid rapeseed oil provided 50 percent of the energy. Microscopic examination of the liver showed fatty change in the hepatocytes of all species from all treatments at seven days of age. This change was most severe in birds fed the HER ration and was found to a lesser degree in ducks and chickens fed this ration at 28 days. The lack of pathological changes in the liver of birds fed diets containing LER is in agreement with previous studies with ducklings (Abdellatif and Vles, I973).

Rats

That rapeseed oil (HER) causes cardiopathogenic changes in rats is now well established, however, even after two months on a rapeseed oil diet, Rocquelin et al. (1970) found no histological lesions in the liver of rats.

In a longer term experiment, Abdellatif and Vles (1973) fed rapeseed oil (HER) for 32 weeks and again found no diet related changes in the liver. An indication of fatty infiltration and degeneration in the central parts of the lobes was noticed after 1 - 2 years (Borg, 1975) however, these changes were not very pronounced. Thus, the pathological effects of rapeseed oil (HER) on the liver of rats is considered to be of minor importance compared with the myocardial alterations.

To date histological techniques have been used to assess pathological changes in heart and liver when diets containing HER and LER were fed. Proliferation of connective tissue could also be followed by using level of hydroxyproline as an index of the amount of collagen present. That level of hydroxyproline in liver may be used as a measure of collagen present and is affected by diet was shown by Feinman and Lieber (1972). They found that incorporation of alcohol in the diets of rats and baboons increased liver levels of hydroxyproline and increased the activity of collagen proline hydroxylase.

From this review of the literature it is apparent that studies on the effect on the liver of feeding rapeseed oil containing diets are limited in the case of the chicken and controversial in the case of the rat. Thus the following studies were conducted to determine the effect that feeding diets containing high and low erucic acid rapeseed

oil has on liver size of chicks, hydroxyproline content of liver protein, lipid content of their liver and fatty acid composition of the liver neutral lipids and phospholipids. For comparative purposes the fatty acid composition of carcass lipid also was studied.

MATERIALS AND METHODS

Duplicate experiments were conducted in which chicks were fed diets containing 20 parts high erucic acid rapeseed oil (HER), 20 parts low erucic acid rapeseed oil (LER) or 20 parts sunflowerseed oil (SFO). The diets were formulated from the high carbohydrate diet (Table 1), by substituting 20 parts oil isoenergetically for glucose. Metabolizable energy values used in formulating the chick diets were 3.64, 7.29, 8.58 and 8.88 kcal/g for glucose, HER, LER and SFO respectively. The composition of the diets fed is shown in Table 2.

In both experiments, each diet was fed ad libitum to quadruplicate groups of ten male crossbred (Dominant White x White Plymouth Rock) chicks from four to twenty-eight days of age. During the first four days of life the chicks were fed the semipurified high carbohydrate diet shown in Table 1. They were then assigned to experimental groups, equalizing both mean body weight and weight distribution among the groups. The chicks were housed in electrically heated, thermostatically-controlled battery brooders with raised wire-screen floors, in a temperature controlled laboratory. Water was supplied ad libitum. Data on growth and feed consumption were obtained weekly.

At the end of each feeding regimen the chicks were killed by cervical dislocation and the livers and

Table 1

Composition of high carbohydrate diet

Ingredients	Percent
Constants	
Soybean meal	35.00
Glycine	1.00
Methionine	0.50
Brewer's yeast	2.50
Dried whey	2.00
Limestone	1.14
Dicalcium phosphate	1.84
Mineral mixture[1]	0.41
Vitamin mixture[2]	0.28
Choline chloride (50%)	0.60
Soybean oil	0.50
Salt	0.60
Antioxidant[3]	0.025
Variables	
Glucose	53.605

[1] Mineral mixture supplied in mg/100g of diet: K_2HPO_4, 220; $MgSO_4$, 115; $FeSO_4 \cdot 7H_2$), 28; $ZnCO_3$, 9.7; $CuSO_4 \cdot 5H_2O$, 0.78; KI, 0.29; $NaSeO_3$, 0.022; $MnSO_4 \cdot H_2O$, 33.5; Cr_2O_3, 300.

[2] Vitamin mixture supplied per 100 mg of diet: thiamin HCl, 1.0mg; riboflavin, 1.0mg; Ca pantothenate, 4.0mg; biotin, 0.04mg; pyridoxine, 2.0mg; niacin, 8.0mg; folacin, 0.3mg; menadione, 0.3mg; aureomycin, 1.0mg; vitamin B_{12}, 0.000005mg; vitamin A palmitate, 1000 I.U.; cholecalciferol, 150 I.U.; alpha tocopherol, 3.3 I.U.

[3] Contains 25% ethoxyquin, Monsanto Chemical Co. St. Louis Missouri, U.S.A.

Table 2

Composition of diets

Oil	Level	Constant ingredients	Glucose[1]	Cellulose[2]	Total
	(g)	(g)	(g)	(g)	(g)
HER[3]	20.00	36.70	4.52	9.00	70.22
LER[4]	20.00	36.70	6.16	9.00	71.86
SFO[5]	20.00	36.70	13.26	9.00	78.96

1 Cerelose

2 Alpha-floc BW-40 Brown Company, Berlin, New Hampshire, U.S.A.

3 High erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

4 Low erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

5 Sunflowerseed oil, "Safflo." Co-op Vegetable Oils Ltd., Altona, Manitoba.

hearts were removed and stored at -29 C until analyzed. After cooling, the contents of the gastrointestinal tract were removed and the residual carcasses from each experimental groups were frozen, ground, mixed and an aliquot dried by lyophilization.

In preparation for analysis the frozen livers were first thawed at refrigerator temperature, ground and mixed in a blender and then freeze dried. Moisture content was determined by loss of weight during freeze drying. The dried samples of liver and carcass were then ground in a blender and transferred to glass jars and stored at -29 C until analyzed.

The carcass samples were analyzed for total lipid content using a Goldfish apparatus and diethyl ether as the solvent. The liver samples were analyzed for total lipid content using a Goldfish apparatus and a mixture of chloroform and methanol (2:1 v/v) as the solvent (Feigenbaum and Fisher, 1963). The total liver lipid was separated into two fractions, triglycerides and phospholipid, using a modification of the method of Hanahan (1957) (see Appendix). The fatty acid composition of the liver and carcass lipid was determined from methyl esters prepared by the method of Metcalfe et al. (1966) and using a gas chromatograph (Bendix 2500, Model 2532-2, Bendix Process Instr. Div., Ronceverte, West Virginia, U.S.A.) equipped with a 300 x 3 mm glass column packed with 10% phenyl-50 cyanopropyl on 80/100 mesh chromosorb

W and a flame ionization detector. Peaks were quantitated by use of digital integrator (model 5300, Spectra Physics, Santa Clara, California) and identified by comparison of retention data with those of known standards (PUFA No. 2 and AOCS Oil Reference Mixture RM-3, Supelco Inc., Bellefonte, Pennsylvania). Fraction by weight of a component in a mixture was calculated by the formula proposed by Eastman (1957). The unsaturated index was calculated as proposed by Richardson et al. (1961): U.I. $= \Sigma_{\alpha=1}^{k}$ (number of double bonds in α) x (wt.% or mole % occurence of α) for each fatty acid α in a group of k fatty acids.

The fatty acid composition of the oils fed was determined by the methods used for analysis of liver and carcass lipid. The fatty acid composition of the oils fed is shown in Table 3.

The hydroxyproline content of the liver samples was determined using a modification of the Neuman and Logan method for the determination of hydroxyproline as proposed by Leach (1960) (see Appendix).

The methods of processing excreta, conducting chemical analyses for moisture, nitrogen and combustible energy and for computing metabolizable energy from the data have been outlined previously. (Hill and Anderson, 1958; Hill et al. 1960; Renner and Hill, 1960). Protein content of the liver samples was determined by multiplying the nitrogen content by 6.25.

Table 3

Fatty acid composition of oils

Fatty acid	Percentage of total fatty acids		
	SFO[1]	HER[2]	LER[3]
16:0	5.6	2.6	3.1
16:1	-	0.2	0.2
18:0	3.2	1.1	1.4
18:1	13.2	25.2	65.5
18:2	77.9	15.3	18.8
18:3	-	6.7	6.7
20:0	-	-	-
20:1	-	11.7	1.8
20:2	-	-	-
20:4	-	0.3	0.0
22:0	-	0.4	0.2
22:1	-	36.5	2.5

1 Sunflowerseed oil, "Safflo" Co-op Vegetable Oils Ltd., Altona, Manitoba.

2 High erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

3 Low erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

The results were analyzed by analysis of variance and treatment differences were evaluated by Duncan's multiple range test (Steele and Torrie, 1960) at the 0.05 level of significance. The number of observations in each experiment was four. When results from both experiments were combined for analysis the number of observations was eight.

RESULTS

Data showing weight gain, energy consumption and energetic efficiency of chicks fed diets containing 20 parts HER, LER or SFO ad libitum are summarized in Table 4. Analyses of variance and application of Duncan's new multiple range test (Steele and Torrie, 1960) to these data showed that when fed ad libitum, chicks fed diets containing HER consumed fewer calories and grew at a slower rate than chicks fed comparable diets containing either LER or SFO. These results also illustrate that chicks fed diets containing LER ate less and grew at a slower rate than SFO fed chicks.

The effect of the incorporation of HER, LER and SFO in the diet of the chick on liver weight and the fat, protein and hydroxyproline content of the liver is shown in Table 5. Analyses of variance and application of Duncan's new multiple range test to these data showed that, when expressed as grams liver per 100 grams body weight, the chicks fed 20 parts HER had significantly heavier livers than the chicks fed 20 parts LER, which in turn, had significantly heavier livers than chicks fed 20 parts SFO. Analyses of the livers for their fat content showed that chicks fed diets containing 20 parts HER had livers containing a significantly higher percentage of fat than the SFO fed controls. The level of fat in livers of chicks fed 20 parts LER was intermediate between that of chicks fed diets containing SFO and HER but was not significantly

Table 4

Weight gain, energy consumption and energetic efficiency of chicks fed the experimental diets ad libitum for 24 days

Diet	Exp. No.	Weight gain		Energy consumption[1]		Energetic efficiency[2]	
		(g)		(Kcal)			
HER[3]	1	748[b]		3670		.204	
	2	732		3670		.199	
			740[a]		3670[a]		.202[a]
LER[4]	1	855		4090		.209	
	2	828		4060		.204	
			842[b]		4075[b]		.206[b]
SFO[5]	1	910		4300		.211	
	2	880		4220		.209	
			895[c]		4280[c]		.210[b]

1 Calculated using determined metabolizable energy values for the diets.

2 Grams gain per kilocalorie of metabolizable energy consumed.

3 High erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

4 Low erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

5 Sunflowerseed oil, "Safflo" Co-op Vegetable Oils Ltd., Altona, Manitoba.

6 Values are averages of quadruplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their superscripts are significantly different ($p < 0.05$).

Table 5

Weight, fat content, protein content and hydroxyproline content of livers of chicks fed experimental diets ad libitum for 24 days

Diet	Exp. No.	Liver size g/100g body weight	LIVER		
			Lipid (% dry matter)	Protein (% dry matter)	Hydroxy-proline (% of protein)
HER[1]	1	2.76[4]	22.8	72.6	.08
	2	2.92	22.5	73.2	.08
		2.84[c]	22.6[b]	72.9[a]	.08[a]
LER[2]	1	2.40	22.2	73.8	.09
	2	2.60	21.2	73.4	.08
		2.50[b]	21.7[ab]	73.6[a]	.08[a]
SFO[3]	1	2.36	20.4	74.5	.08
	2	2.37	21.1	74.5	.08
		2.36[a]	20.7[a]	74.5[a]	.08[a]

1 High erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

2 Low erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

3 Sunflowerseed oil, "Safflo" Co-op Vegetable Oils Ltd., Altona, Manitoba.

4 Values are averages of quadruplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their superscripts are significantly different ($p < 0.05$).

different from either. Statistical analyses of the data on the protein and hydroxyproline content of the livers of chicks fed the experimental diets revealed no significant differences between treatments.

The phospholipid content of the livers of chicks fed the experimental diets is shown in Table 6. For comparative purposes total fat content is also given. Statistical analyses revealed that, although chicks fed 20 parts HER had a significantly higher percentage of liver fat than chicks fed 20 parts SFO, the phospholipid content, expressed as a percentage of total fat, did not differ between treatments.

The fatty acid composition of liver neutral lipid of chicks fed diets containing HER, LER and SFO are summarized in Table 7, 8 and 9. For comparative purposes the fatty acid composition of carcass lipid is also given. A three-way analysis of variance was conducted to test the significance of the main effects - type of fat, type of tissue and experiment number - and their interactions on the fatty acid composition of liver neutral lipid and carcass lipid. A summary of the mean squares obtained for selected fatty acids from these analyses is found in Table 10. Duncan's new multiple range test was used to determine how the fatty acid composition of liver neutral lipid and carcass lipid was affected by feeding diets containing HER, LER and SFO.

Table 6

Fat content and phospholipid content of livers of chicks fed experimental diets ad libitum for 24 days

Diet	Exp. No.	Fat		Phospholipid	
		(% of dry matter)		(% of total fat)	
HER[1]	1	22.8[4]		74.8	
	2	22.5		76.9	
			22.6[b]		75.8[a]
LER[2]	1	22.2		75.8	
	2	21.2		76.0	
			21.7[ab]		75.9[a]
SFO[3]	1	20.4		74.2	
	2	21.1		79.3	
			20.7[a]		76.8[a]

1 High erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

2 Low erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

3 Sunflowerseed oil, "Safflo" Co-op Vegetable Oils Ltd., Altona, Manitoba.

4 Values are averages of quadruplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their superscripts are significantly different ($p < 0.05$).

Table 7

Saturated fatty acid content of liver neutral lipids and carcass fat of chicks fed experimental diets ad libitum for 24 days

Diet	Exp. No.	Percent of total fatty acids SFA		16:0		18:0	
Carcass lipid							
HER[1]	1	9.9[4]		6.8		3.1	
	2	8.6	9.3[b]	6.0	6.4[b]	2.7	2.9[ab]
LER[2]	1	8.2		5.5		2.7	
	2	7.3	7.8[a]	4.8	5.2[a]	2.5	2.6[a]
SFO[3]	1	12.4		7.9		4.4	
	2	11.5	11.9[c]	7.0	7.5[d]	4.5	4.4[bc]
Liver neutral lipid							
HER	1	12.0		6.8		5.2	
	2	11.8	11.9[c]	6.5	6.6[bc]	5.3	5.2[cd]
LER	1	12.5		6.8		5.7	
	2	14.8	13.6[d]	7.6	7.2[cd]	7.1	6.4[d]
SFO	1	17.8		9.0		8.8	
	2	19.9	18.9[e]	9.8	9.4[e]	10.1	9.5[e]

1 High erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

2 Low erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

3 Sunflowerseed oil, "Safflo" Co-op Vegetable Oils Ltd., Altona, Manitoba.

4 Values are averages of quadruplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their superscripts are significantly different ($p < 0.05$).

Table 8

N-9 fatty acid content of liver neutral lipid and carcass fat of chicks fed experimental diets ad libitum for 24 days

Diet	Exp. No.	Percent of total fatty acids N-9		18:1		20:1		22:1	
Carcass lipid									
HER[1]	1	69.1[4]		47.3		11.1		10.8	
	2	66.1	67.6[cd]	45.1	46.2[b]	10.8	10.9[c]	10.3	10.5[c]
LER[2]	1	73.2		71.8		1.2		0.2	
	2	67.2	70.2[d]	66.0	68.9[e]	1.2	1.2[a]	-	0.1[a]
SFO[3]	1	17.2		17.2		-		-	
	2	16.2	16.7[a]	16.2	16.7[a]	-		-	
Liver neutral lipid									
HER	1	65.0		46.5		9.1		9.4	
	2	67.6	66.3[c]	50.8	48.6[c]	8.9	9.0[b]	8.0	8.7[b]
LER	1	60.9		59.6		1.3		-	
	2	60.0	60.5[b]	58.7	59.2[d]	1.4	1.3[a]	-	
SFO	1	15.0		15.0		-		-	
	2	15.9	15.5[a]	15.9	15.5[a]	-		-	

1 High erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

2 Low erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

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4 Values are averages of quadruplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their superscripts are significantly different ($p < 0.05$)

Table 9

Unsaturated fatty acid index and polyunsaturated fatty acid of liver neutral lipid and carcass fat of chicks fed experimental diets ad libitum for 24 days

Diet	Exp. No.	Unsaturated index		18:2		18:3		20:4	
Carcass fat									
HER[1]	1	113[4]		17,0		2.9		-	
	2	119	116[ab]	19.7	18.3[a]	4.4	3.7[d]	-	
LER[2]	1	112		15.5		2.2		-	
	2	121	116[ab]	20.8	18.2[a]	3.8	3.0[c]	-	
SFO[3]	1	158		69.7		.43		-	
	2	160	159[d]	72.0	70.8[d]	-	0.2[a]	-	
Liver neutral lipid									
HER	1	113		18.0		2.8		0.6	
	2	112	113[a]	16.7	17.3[a]	2.3	2.6[b]	0.6	0.6[a]
LER	1	120		20.6		3.6		1.6	
	2	115	118[b]	20.2	20.4[b]	2.6	3.1[c]	1.4	1.5[b]
SFO	1	154		65.0		-		2.1	
	2	147	150[c]	62.3	63.7[c]	-		1.6	1.8[b]

1 High erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

2 Low erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

3 Sunflowerseed oil, "Safflo" Co-op Vegetable Oils Ltd., Altona, Manitoba.

4 Values are averages of quadruplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their subscripts are significantly different ($p < 0.05$).

Table 10

Variance analysis for effects of dietary fats, liver neutral lipid and carcass lipid and experiment

Source of variation	DF	Mean squares									
		16:0	18:0	18:1	18:2	20:1[1]	20:4	22:1[2]	SFA	N-9	Unsat. index
Fat	2	23.9**	38.4**	9478**	12654**	607.1**	1.48**	-	120**	13380**	8210**
Tissue	1	23.9**	166.7**	96.5**	45.8**	6.53	20.7**	12.3**	316**	201**	173**
Experiment	1	0.46	1.66*	8.16	12.1	0.07	0.15	2.84	0.37	19.0*	8.87
Fat x tissue	2	3.97**	7.16**	155**	91.5**	8.12**	1.48**	-	20.2	95.7**	103**
Fat x experiment	2	0.55	0.80	21.0**	7.47	0.18	0.09	-	2.63	14.6**	30.2
Tissue x experiment	1	5.15**	3.70**	58.1**	72.3**	0.02	0.15	1.59	17.6	53.4**	345**
Fat x tissue x experiment	2	0.50	0.36	5.40	0.72	0.003	0.09	-	6.20	4.16	10.1
Error	36	0.45	0.36	3.99	4.83	0.39	0.12	0.64	1.17	4.35	16.9

1 Sources of variation and degrees of freedom for 20:1 were: fat, 1; tissue, 1; experiment, 1; fat x tissue, 1; fat x experiment, 1; tissue x experiment, 1; fat x tissue x experiment, 1; error, 24.

2 Sources of variation and degrees of freedom for 22:1 were: tissue, 1; experiment, 1; tissue x experiment, 1; error, 12.

Results summarized in Table 7 show that carcass lipid and liver neutral lipid of chicks fed diets containing HER and LER were significantly less saturated than carcass and liver neutral lipids of chicks fed SFO. This difference was due to markedly lower levels of palmitic and stearic acids when HER and LER were fed than when SFO was fed. The data also show that the saturated fatty acid content of carcass lipid of chicks fed diets containing HER was significantly greater than when LER was incorporated in the diet, while the liver neutral lipids of chicks fed HER were less saturated than those of chicks fed diets containing LER. Similar trends were observed for palmitic and stearic acids, but in most cases the differences were not great enough to be significant.

That liver neutral lipids are significantly more saturated than carcass lipid when HER, LER or SFO was incorporated in the diet is shown by the data summarized in Table 7. Higher levels of stearic acid in liver neutral lipids was the major factor contributing to the greater saturation of liver neutral lipids as compared to carcass lipid. Comparison of the level of saturated fatty acids in the oils fed, as shown in Table 4, with the saturated fatty acid content of carcass lipid and liver neutral lipids shows that carcass lipid and liver neutral lipids are much more saturated than the oils fed.

The effect of type of fat fed on the monounsaturated fatty acid composition of carcass lipid and liver neutral lipids is shown by the data summarized in Table 8. The data show that carcass lipid and liver neutral lipid of chicks fed diets containing HER and LER had higher levels of N-9 fatty acids than carcass lipid and liver neutral lipids of chicks fed diets containing SFO. This was reflected in higher levels of oleic, eicosenoic and erucic acids in liver neutral lipids and carcass lipid when HER and LER were fed than when SFO was fed. Results also show that chicks fed HER and LER deposited similar amounts of N-9 fatty acids into carcass lipid, but in the case of liver neutral lipids, chicks fed HER had significantly higher levels of N-9 fatty acids than LER fed chicks. The marked difference in N-9 fatty acid content of carcass lipid and liver neutral lipid of chicks fed diets containing LER appears to be due to a decreased level of oleic acid in liver neutral lipids as compared to carcass lipid. Comparing the level of erucic acid in the HER fed (Table 3) with the erucic acid content of carcass lipid and liver neutral lipids shows that lipids deposited in the liver and carcass are markedly lower in erucic acid than the oils fed. The data also show that liver neutral lipids contained significantly less erucic acid than carcass lipid.

Carcass lipid and liver neutral lipids of chicks fed HER and LER had significantly lower unsaturated

indexes than the carcass lipid and liver neutral lipids of chicks fed SFO as shown in Table 9. This was reflected in the lower levels of linoleic when HER and LER were fed compared to when SFO was fed. The data also show that the unsaturated index and linoleic acid content of carcass lipid of chicks fed HER and LER were similar while the liver neutral lipid of chicks fed HER had a significantly lower unsaturated index and contained less linoleic and arachidonic acids that those of chicks fed diets containing LER.

Summarized in Tables 11, 12 and 13 are data showing the fatty acid composition of liver phospholipids of chicks fed diets containing 20 parts HER, LER and SFO. For comparative purposes the fatty acid composition of liver neutral lipid is also given. A three way analysis of variance was conducted to test the significance of the main effects - type of fat, type of tissue and experiment number - and their interactions on the fatty acid composition of liver neutral lipids and liver phospholipid. A summary of the mean squares obtained for selected fatty acids from these analyses is found in Table 14. Duncan's new multiple range test was used to determine how the fatty acid composition of liver neutral lipids and liver phospholipids was affected by feeding diets containing HER, LER or SFO.

Results summarized in Table 11 show that liver neutral lipids and phospholipids of chicks fed diets

Table 11

Saturated fatty acid content of liver neutral lipid and liver phospholipid of chicks fed experimental diets ad libitum for 24 days

Diet	Exp. No.	Percent of total fatty acids					
		SFA		16:0		18:0	
Liver neutral lipid							
HER[1]	1	12.0[4]		6.8		5.2	
	2	11.8	11.9[a]	6.5	6.6[a]	5.3	5.2[a]
LER[2]	1	12.5		6.8		5.7	
	2	14.8	13.6[a]	7.6	7.2[a]	7.1	6.4[a]
SFO[3]	1	17.8		9.0		8.8	
	2	19.9	18.9[b]	9.8	9.4[b]	10.1	9.5[b]
Liver phospholipid							
HER	1	40.6		12.2		28.3	
	2	41.3	40.9[c]	12.3	12.3[d]	29.0	28.7[c]
LER	1	43.3		11.2		32.1	
	2	47.8	45.5[d]	12.1	11.6[d]	35.7	33.9[d]
SFO	1	48.8		10.5		38.4	
	2	49.8	49.3[e]	11.0	10.7[c]	38.8	38.6[e]

1 High erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

2 Low erucic acid rapeseed oil. Western Canadian Seed Processors Ltd., Lethbridge, Alberta.

3 Sunflowerseed oil, "Safflo" Co-op Vegetable Oils. Ltd., Altona, Manitoba.

4 Values are averages of quadruplicate groups. Underlined values are averages of duplicate experiments. Values without a common letter in their superscripts are significantly different ($p < 0.05$).

Table 12

N-9 fatty acid content of liver neutral lipid and liver phospholipid of chicks fed experimental diets ad libitum for 24 days

Diet	Exp. No.	Percent of total fatty acids N-9		18:1		20:1		22:1	
Liver neutral lipid									
HER[1]	1	65.0[4]		46.5		9.1		9.4	
	2	67.6	66.3[e]	50.8	48.6[d]	8.9	9.0[d]	8.0	8.7[b]
LER[2]	1	60.9		59.6		1.3		-	
	2	60.0	60.5[d]	58.7	59.2[e]	1.4	1.3[b]	-	
SFO[3]	1	15.0		15.0		-		-	
	2	15.9	15.5[b]	15.9	15.5[b]	-		-	
Liver phospholipid									
HER	1	28.3		20.3		5.2		2.8	
	2	27.2	27.8[c]	20.2	20.3[c]	4.8	5.0[c]	2.1	2.5[a]
LER	1	20.2		19.1		0.9		-	
	2	16.1	18.1[b]	15.4	17.3[b]	0.4	0.6[a]	-	
SFO	1	3.3		3.0		-		-	
	2	2.4	2.8[a]	2.1	2.6[a]	-		-	

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Table 13

Unsaturated fatty acid index and polyunsaturated fatty acid content of liver neutral lipids and liver phospholipids of chicks fed experimental diets ad libitum for 24 days

Diet	Exp. No.	Percent of total fatty acids							
		Unsaturated index		18:2		18:3		20:4	
Liver neutral lipid									
HER[1]	1	113[4]		18.0		2.8		0.6	
	2	112	113[ab]	16.7	17.3[a]	2.3	2.6[b]	0.6	0.6[a]
LER[2]	1	120		20.6		3.6		1.5	
	2	115	118[b]	20.2	20.4[b]	2.6	3.0[c]	1.4	1.5[ab]
SFO[3]	1	154		65.0		-		2.1	
	2	147	150[d]	62.3	63.7[e]	-	-	1.6	1.8[b]
Liver phospholipid									
HER	1	109		20.9		0.6		9.0	
	2	109	109[a]	21.1	21.0[bc]	0.3	0.4[a]	9.7	9.3[c]
LER	1	119		22.4		0.8		12.7	
	2	113	116[ab]	22.8	22.6[c]	0.1	0.4[a]	12.8	12.7[d]
SFO	1	131		31.5		0.7		15.9	
	2	130	130[c]	30.5	31.0[d]	-	0.4[a]	16.7	16.3[e]

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Table 14

Variance analysis for effects of dietary fats, liver neutral lipids and liver phospholipids and experiment

Source of variation	DF	Mean squares									
		16:0	18:0	18:1	18:2	20:1[1]	20:4	22:1[2]	SFA	N-9	Unsat. index
Fat	2	2.28**	201**	4047**	3916**	291**	66.6**	-	239**	6412**	3946**
Tissue	1	175**	8542**	9201**	963**	44.1**	1579**	154**	11133**	11668**	860**
Experiment	1	3.36*	18.3*	0.03	7.62*	0.46	0.32	4.58*	35.2*	4.12	117**
Fat x tissue	2	20.0**	34.7**	836**	1688**	22.3**	34.0**	-	8.11*	1046**	407**
Fat x experiment	2	1.52*	4.93	18.7	3.75	0.02	0.15	-	10.4	11.5	23.8
Tissue x experiment	1	0.002	1.14	24.4	5.12	0.29	1.76	0.65	2.01	24.8	23.2
Fat x tissue x experiment	2	0.14	2.33	1.71	0.22	0.07	0.30	-	9.34	1.12	10.9
Error	36	0.49	3.51	5.89	1.59	0.28	1.09	0.84	5.68	8.46	26.6

[1] Sources of variation and degrees of freedom for 20:1 were: fat, 1; tissue, 1; experiment, 1; fat x tissue, 1; fat x experiment, 1; tissue x experiment, 1; fat x tissue x experiment, 1; error, 24.

[2] Sources of variation and degrees of freedom for 22:1 were: tissue, 1; experiment, 1; tissue x experiment, 1, error, 12.

containing HER and LER were significantly less saturated than liver neutral lipids and phospholipids of chicks fed diets containing SFO. This was reflected in significantly lower levels of palmitic and stearic acids in liver neutral lipids when either HER or LER was substituted for SFO in the diet. Significant reductions in stearic acid contributed to the reduction in level of saturated fatty acids in liver phospholipids when HER or LER replaced SFO in the diet. In contrast, level of palmitic acid in liver phospholipids was significantly increased when HER and LER rather than SFO was present in the diet.

Comparison of the fatty acid composition of liver lipids of chicks fed diets containing HER and LER showed that type of rapeseed oil did not affect level of saturated fatty acids in liver neutral lipid, however, in the case of liver phospholipid more stearic acid was incorporated when LER was fed than when HER was fed, which was reflected in greater saturation of liver phospholipids in LER than HER fed chicks.

Comparison of the saturated fatty acid content of the oils fed (Table 3) with the saturated fatty acid content of liver lipids shows liver lipids to be more saturated with liver phospholipids containing significantly more saturated fatty acids than liver neutral lipids, irrespective of type of oil fed.

The effect of type of fat fed on the monounsaturated fatty acid composition of liver neutral lipids

and liver phospholipid is shown in Table 12. The data show that liver neutral lipid and phospholipids of chicks fed diets containing HER and LER had higher levels of N-9 fatty acids than liver neutral lipids and phospholipids of chicks fed SFO. The major factors contributing to this difference were higher levels of oleic, eicosenoic and erucic acid when HER was fed and higher levels of oleic and eicosenoic acids when LER was fed. The results also show that the N-9 fatty acid content of liver neutral lipids and phospholipids from chicks fed HER was significantly greater than when LER was incorporated into the diet. This difference was reflected in significantly higher levels of eicosenoic acid and the presence of erucic acid in the liver neutral lipids and phospholipids when HER was substituted for LER in the diet.

That liver neutral lipids contain significantly higher levels of N-9 fatty acids than liver phospholipids irrespective of whether HER, LER or SFO was incorporated in the diet is shown by the data summarized in Table 12. A higher level of oleic acid was the major factor contributing to the greater monounsaturation of liver neutral lipids as compared to liver phospholipids.

Comparison of the level of erucic and eicosenoic acids in the HER fed (Table 3) and the level of these acids deposited in liver neutral lipids and liver phospholipid shows a progressive decrease with oil containing

a higher level of erucic and eicosenoic acid than liver neutral lipids which in turn contain a higher level than liver phospholipids.

Data summarized in Table 13 showed that liver neutral lipids and phospholipids of chicks fed diets containing HER and LER had lower unsaturated indexes and contained lower levels of both linoleic and arachidonic acids than when diets containing SFO were fed.

Comparison of the fatty acid composition of liver lipids of chicks fed diets containing HER and LER showed that level of arachidonic acid in liver phospholipids was significantly lower when HER was fed than when LER was fed, however, this difference was not great enough to cause a significant depression in unsaturation index. In the case of liver neutral lipids substitution of HER for LER in the diet reduced level of linoleic acid significantly but did not affect level of arachidonic or unsaturated index, significantly. The data also show that regardless of the type of fat fed chicks deposited significantly higher levels of arachidonic acid into liver phospholipid as compared to liver neutral lipid.

DISCUSSION

The finding that the inclusion of 20 parts HER in the diet of the chick depressed growth at 24 days is in agreement with results reported for the chick by Clement and Renner (1977), Vogtmann et al. (1973), Sell and Hodgson (1962) and Kramer and Hulan (1977); and for the rat by Beare et al. (1957), Rocquelin and Cluzan, (1968) and Kramer et al. (1973). Since the decreased growth rate was accompanied by a decrease in consumption, it would appear that HER depresses growth by decreasing appetite. This is in agreement with Beare et al. (1959b) who observed that differences in rate of growth of rats largely disappeared when body weight gains were adjusted for food consumption by covariance analysis. The reason for the depression of appetite is unknown; however, since chicks fed diets containing 20 parts HER utilized energy less efficiently than chicks fed diets containing SFO, they must be losing more energy as heat. Whether increased heat production stimulates the satiety center and reduces food intake is unknown. Support for this concept has been provided by Hornstra (1972). He observed that intubation of rapeseed oil stimualted greater basal oxygen consumption in rats than sunflowerseed oil and suggested that ingestion of rapeseed oil may lead to hypothermia.

Results showed that chicks fed diets containing

LER grew significantly faster than chicks fed diets containing HER, but significantly slower than chicks fed diets containing SFO. Previously, Clement and Renner (1977) found LER to be equal to SFO in promoting growth of chicks. Walker et al. (1970) and Vogtmann et al. (1973) also have reported that chicks fed LER at a level of 20% and 15% of the diet, respectively, grew at a similar rate to chicks fed tallow and soybean oil, respectively. In rats, Abdellatif and Vles (1970b), Rocquelin et al., (1970), Craig and Beare (1968) and Kramer et al. (1973) found that diets containing 15 or 20% by weight of low erucic acid rapeseed oil promoted the same weight gain as diets containing similar levels of sunflowerseed oil, peanut oil, olive oil, or corn oil, respectively. The reason why LER promoted a slower rate of growth in these experiments is unknown.

The inclusion of 20 parts rapeseed oil in the diet of the chick decreased energetic efficiency when grams gain per kilocalorie consumed was used as the criterion of efficiency. Hornstra (1972) also observed lower weight gains per unit of digestible energy consumed when he fed rats diets containing 31.5% rapeseed oil. Hornstra (1972) also showed that oxygen consumption and water vapour loss of rapeseed oil fed rats was consistently higher than that of rats fed the sunflowerseed oil containing diet. He suggested that the lower efficiency of the rapeseed oil diet was caused by a slightly uncoupled oxidative phosphorylation and a greater heat increment.

Other evidence that rapeseed oil may interfere with energy metabolism in at least some tissues of the rat is the finding of Houtsmuller et al. (1970) that the feeding of diets containing 50 cal percent of energy as rapeseed oil caused the mitochondria of the heart, but not of the liver, to malfunction. They concluded that dietary erucic acid causes a considerable decrease in the capacity of heart mitochondria to oxidize substrates. Results from in vitro studies with isolated rat heart and liver mitochondria have been conflicting. Christopherson and Bremer (1972) concluded from their studies that erucic acid or one of its metabolites has an inhibitory effect on the beta-oxidation of other fatty acids. Swarttouw (1974), on the other hand, could not find inhibition of the oxidation of other fatty acids by erucic acid although she found, in agreement with Kramer et al. (1973), that the oxidation of erucic acid itself is much lower compared to that of other fatty acids. Dow-Walsh et al. (1975) also investigated the effect of dietary erucic acid on the ability of isolated rat heart mitochondria to produce ATP and concluded that there was no decrease in ATP production in the hearts of rapeseed oil fed rats but suggested a possible impairment of ATP utilization due to changes in membrane fatty acid composition. Whether the decreased energetic efficiency observed in chicks fed high erucic acid rapeseed oil containing diets was due to malfunctioning of the mitochondria and/or uncoupling of oxidative phosphorylation is unknown although

recent studies (Renner et al., 1979) have indicated that a complex dynamic mechanism associating dietary fat with energetic efficiency and mitochondrial structural functional transitions exists in the growing chick.

The finding that chicks fed diets containing 20 parts HER for 24 days had significantly heavier livers, when expressed as a percentage of body weight, than chicks fed diets containing 20 parts SFO is in agreement with results reported by Sheppard et al. (1971), Vogtmann et al. (1974), and Bragg et al. (1973). In rats, results are conflicting. Beare et al. (1959b) and Abdellatif and Vles (1973) found that rats fed diets containing rapeseed oil had significantly heavier livers than rats fed control oils while Rocquelin et al. (1970) reported no significant increase in liver weight. These differences might be attributed to the fact that the level of rapeseed oil added to the diets was not the same in all cases.

Results of these experiments also showed that chicks fed diets containing 20 parts HER for 24 days had significantly heavier livers, when expressed as a percentage of body weight, than chicks fed diets containing 20 parts LER. This is in agreement with Vogtmann et al. (1974) and Ratanasethkul et al. (1976). In rats, on the other hand, Rocquelin et al. (1970) found no significant difference in liver weights between rats fed diets containing 15% rapeseed oil and 15% Canbra oil.

The results also showed that livers of chicks fed diets containing HER contained significantly more fat than did chicks fed diets containing 20 parts SFO. This is in agreement with Bragg et al. (1973). Sheppard et al. (1971), however, found no difference in the fat content of liver when chicks were fed diets containing 16% rapeseed oil or 16% corn oil for three weeks. Whether differences would have become significant had the fat content been expressed as percent dry matter is unknown. Vogtmann et al. (1974) also found that the total lipid content of liver was not influenced by the kind of oil or fat included in the rations of chicks fed for 28 days. In rats, results are also conflicting. Kramer et al. (1973) and Kienle et al. (1976) found that total lipids were significantly higher in the livers of rapeseed oil fed animals whereas Beare et al. (1957), Rocquelin et al. (1970), Houtsmuller et al. (1970) and, in a subsequent experiment that same year, Kramer (I973) reported no significant difference in liver fat content between rats fed rapeseed oil diets and control animals. The findings of this experiment would suggest that the increase in liver weights of chicks fed 20 parts HER may be due, at least in part, to an increase in fat content of the liver.

Although liver lipid increased in chicks fed diets containing HER, there was no apparent alteration in the amount of phospholipid in the liver. This is in agreement with Beare-Rogers et al. (1971), Kramer et al.

(1973) and Kienle et al. (1976) using the rat as the experimental animal. It is probable that the increased fat content was due to increased levels of neutral lipid in the liver, however, the analytical technique employed in this study did not permit quantitative measurement of the amount of neutral lipid in the liver. Support for this suggestion was obtained by Kienle et al. (1976) who found that the level of liver triglyceride in rats fed a 25% rapeseed oil diet was significantly higher than the level of liver triglycerides in rats fed diets containing 25% olive oil.

The observation that chicks fed diets containing HER had significantly heavier livers containing a higher proportion of fat than did chicks fed diets containing SFO suggested that connective tissue also might be increased. Analysis of the livers for hydroxyproline showed that the percentage of hydroxyproline in the protein was similar irrespective of whether the diet contained HER, LER or SFO. Since hydroxyproline is only found in collagen and as collagen is the chief constituent of connective tissue these results indicate that the increase in weight of livers of chicks fed diets containing HER is not due to connective tissue. That level of hydroxyproline in liver may be used as a measure of collagen present and is affected by diet was shown by Feinman and Lieber (1972). They found that incorporation of alcohol in diets of rats and baboons increased liver levels of hydroxyproline and increased

the activity of collagen proline hydroxylase.

The ability of the chick to modify dietary fat prior to incorporation into liver and carcass lipid is evident from comparisons of the fatty acid composition of the oils fed and the fatty acid composition of the liver neutral lipid, carcass lipid and liver phospholipids. In general, dietary fatty acids were observed to have more influence on the fatty acid composition of the carcass lipid and liver neutral lipid than on the liver phospholipids. This finding is not surprising since one of the major functions of phospholipids is as constituents of membranes which would have a relatively constant composition. In the case of chicks fed HER, the greatest differences between oils fed and liver neutral lipid and carcass lipid deposited were observed in the marked increases in proportion of palmitic, stearic and oleic acids and the marked decrease in the level of erucic acid. In the case of LER, liver neutral lipids and carcass lipid of chicks contained greater proportions of palmitic and stearic acids and a lower proportion of erucic acid when compared to the oil fed. Overall, the tissues of chicks fed rapeseed oils were more saturated and contained lower levels of N-9 fatty acids than the oils fed.

Essentially similar results were reported for the chick by Sim et al. (1973) who found that the level of saturated fatty acids increased from 7% in the oil fed

to 31.4% and 23.4% in the total liver and carcass lipids, respectively, when a diet containing 8% HER was fed to laying hens for 28 days. These authors suggested that possibly a homeostatic mechansim exists in the liver which stimulates the synthesis of saturated fatty acids during a period of unsaturated fatty acid ingestion in order to maintain a specific ratio of unsaturated to saturated fatty acids. In experiments using the hen as the experimental animal, Leclerq (1972) found that the level of palmitic acid increased from 7.74% in the oil fed to 23.6% in total liver lipids when diets containing 5% HER were fed for one month. He also found that the level of stearic acid increased from 2.97% in the oil fed to 13.4% in the total liver lipid. In rats, Craig et al. (1963b) also found that levels of saturated fatty acids in liver glycerides and carcass lipid were greater than in the oil fed when a diet containing 20% HER was fed for 21 weeks. They also reported that the level of palmitic acid in the liver glycerides and carcass lipid was similar to the oil fed while the level of oleic acid was increased from 18.8% in the oil fed to 55.2% and 42.1% in the liver glycerides and carcass lipid, respectively.

Results also showed that a marked decrease in erucic acid, in comparison to the oil fed, occurred in the livers and carcasses of chicks fed diets containing HER. Similar results have been reported in studies using the laying hen as the experimental animal (Leclerq, (1972),

Sim et al., (1973)). Studies with rats have also shown that levels of erucic acid in liver lipid (Craig et al. (1963b), Kramer (1973), Walker (1972), Beare (1961)) and in carcass lipid (Craig et al. (1963b) Beare (1961)) were much lower than in the oil fed. In all cases, the decreases in erucic acid were accompanied by increased levels of oleic acid lending support to the suggestion made by Craig et al. (1963b) that erucic acid is converted to oleic acid in the liver by B-oxidation.

Comparison of the erucic and eicosenoic acid content of carcass lipid and liver neutral lipids of chicks fed diets containing HER showed that liver neutral lipids contained slightly but significantly lower levels of these fatty acids than carcass lipid. Differences in levels of erucic and eicosenoic acids in carcass lipid and liver neutral lipids of rats fed diets containing HER were much greater (Craig et al. 1963b). They found levels in carcass lipid and liver neutral lipid to be 6.9 and 1.8%, respectively, for erucic acid and 9.5 and 2.9%, respectively, for eicosenoic acid. Although in this experiment erucic and eicosenoic acid content of heart lipids was not determined, Clement and Renner (1977) found levels of these fatty acids to be slightly but significantly lower in heart than in carcass lipids. In comparison, in rats erucic and eicosenoic acid content of heart lipid has been found to be markedly higher than levels in liver and carcass in short term experiments

(Beare-Rogers et al. 1972; Kramer et al. 1973), with the difference persisting although of smaller magnitude in long term experiments (Kramer et al. 1973).

The finding that in chicks the levels of erucic and eicosenoic acid in carcass, heart and liver neutral lipids showed less variation between tissues than has been reported for the rat may reflect differences in fat metabolism between rats and chicks.

One major difference in fat metabolism between chicks and rats is in the route of absorption of long chain fatty acids. Studies have shown that in the chick the major route of absorption is via the portal system (Noyan et al. 1964) while in the case of the albino rat long chain fatty acids are absorbed via the lymphatic system. Another difference between fat metabolism in chicks and rats lies in the site of fatty acid biosynthesis. Studies have shown (O'Hea and Leveille, 1969; Leveille et al. 1975) that the liver is probably the most important site of lipogenesis in the chick. Their studies suggest that fatty acids are synthesized in the liver and transported in the low density or B-lipoprotein fraction (O'Hea and Leveille, 1969) to the adipose tissue for storage. In contrast, studies in rats have shown that over 50% of the fatty acids are synthesized in the adipose tissue (Leveille, 1976; Leveille et al. 1975). The central role which the liver plays in fat metabolism in the chick may increase its exposure to erucic and eicosenoic

acids while reducing the exposure of other tissues. In this regard studies have shown that morphological changes in the heart and skeletal muscle are less marked in chicks (Lall et al. 1972) as compared to rats while livers of chicks may be more adversely affected than livers of rats (Ratanasethkul et al., 1976). Since recent studies (Galton, 1968; Shrago et al., 1969, 1971) indicate that in the human, the liver is also the major site of fatty acid biosynthesis, the question arises as to whether type of fatty acid ingested would affect the liver more than other tissues in humans. Since absorption of long chain fatty acids in the human is via the lymphatic system the liver may not reduce exposure of other tissues to erucic and eicosenoic acid to the same extent as in the chicken.

If the hypothesis that in the chick the liver helps to protect other tissues from excessive exposure to erucic and eicosenoic acids is true, then the question arises as to why levels of erucic and eicosenoic acids are slightly but significantly higher in carcass than in liver neutral lipids when chicks were fed diets containing HER. If chicks, like rats, oxidize erucic acid at a slower rate than oleic acid (Carroll, 1966), this would contribute to higher levels of erucic acid in carcass lipid than in liver neutral lipids. Also, if chicks, like rats, oxidize palmitic acid to carbon dioxide more rapidly than erucic acid (Carroll, 1962) then this may explain why carcass lipids contained less palmitic acid

than did liver neutral lipids.

Differences also exist in the fatty acid composition of liver neutral lipids and liver phospholipid. Results showed that chicks fed HER or LER had higher levels of palmitic, stearic, linoleic and arachidonic acids but lower levels of N-9 fatty acids in liver phospholipids than in liver neutral lipids. In rats, Craig et al. (1963b) also reported higher levels of palmitic and stearic acids in liver phospholipids when diets containing HER were fed, but in contrast to the chick, they found higher levels of linoleic acid in the glyceride portion of the liver. They also found high levels of behenic acid in the liver phospholipids of rats fed HER while, in the chick, this fatty acid did not appear in the liver phospholipids. Similarly, Kramer (1973) reported higher levels of stearic acid in liver phospholipids but levels of palmitic and linoleic acids were found to be lower in the phospholipid than in the triglyceride fraction of liver lipids from rats fed HER.

When compared to the liver phospholipids from chicks fed SFO, the liver phospholipids from chicks fed HER or LER were significantly less saturated and contained significantly lower levels of linoleic and arachidonic acids. It has been suggested by Craig and Beare (1968) that the predominantly unsaturated fatty acids of rapeseed might decrease the supply of saturated fatty acids required for the alpha position of phospholipids. If the level of saturated fatty acids in rapeseed oil fed chicks is

critically low for the synthesis of these phospholipid components of membranes, a weakening of the membranes resulting in a reduction of functional capacity could be expected.

Erucic acid was incorporated into both liver lipid fractions of chicks fed diets containing HER, with higher levels deposited in the liver neutral lipids. Kramer (I973) also reported that erucic acid was deposited in liver triglycerides and phospholipids from rats fed HER but levels were similar in both fractions. In contrast, Craig et al. (I963b) found that erucic acid was incorporated into the glycerides but not into the phospholipids of livers of rats fed HER containing diets.

In chicks fed diets containing HER or LER, eicosenoic acid was deposited to a greater extent in the liver neutral lipids than in liver phospholipids. In rats, Craig et al. (I963b) found a greater deposition of eicosenoic acid in liver phospholipids than in liver neutral lipids of rats fed HER diets, while Kramer (I973) found similar levels of eicosenoic acid in liver triglyceride and liver phospholipid.

Whether the changes in fatty acid composition of liver phospholipids in chicks fed rapeseed oils impair mitochondrial function is unknown. It has recently been shown (Divakaran and Venkataraman, I977) that liver mitochondria from rats fed coconut oil with a low level of polyunsaturated fatty acids had lower P:O ratios than those

from rats fed safflower oil with a high level of polyunsaturated fatty acids. Analyses of the liver phospholipids and individual mitochondrial phospholipids showed significantly lower levels of polyunsaturated fatty acids in the rats fed coconut oil. These authors suggested that the changes in the phosphorylation capacity of the liver mitochondria were due to the changes in the mitochondrial lipids which were a reflection of the dietary fat. Green and Fleischer (I963) and Pullmann and Schatz (I967) have proposed that the uncoupling of the oxidative phosphorylation may be due to a molecular defect caused by the absence of essential fatty acids in the structure that determines the spatial relationship between the electron transport chain and the oxidative phosphorylation.

Little work has been done on the effect of rapeseed oil feeding on the oxidative phosphorylation of chick liver mitochondria, however, in the hearts of chics fed HER it has recently been shown (Renner et al., I979) that rapeseed oil does modify the fatty acid composition of cardiac mitochondrial membranes and that the cardiac mitochondria isolated from chicks fed HER had significantly reduced ADP/O ratios and reduced rates of ATP synthesis utilizing pyruvate and malate as the respiratory substrates when compared with mitochondria isolated from chicks fed SFO. In particular, Renner et al. (I979) found that the fatty acid composition of diphosphatidylglycerol, known to have functions integral to processes of oxidative phosphorylation and membrane functions in electron transport

(Awasthi et al., 1970; Awasthi et al., 1971; Santiago et al., 1973) was markedly affected by dietary rapeseed oil feeding. The fatty acid composition of this phospholipid was characterized by decreased levels of linoleic acid and corresponding increased levels of oleic, eicosenoic and erucic acid for chicks fed diets containing HER or LER when compared to chicks fed SFO containing diets. Results obtained in the present study of the fatty acid composition of the total liver phospholipids showed a similar pattern. It is therefore possible that dietary induced changes in liver phospholipid composition in the chicks may cause an impairment of mitochondrial function in the liver.

Other observations which support the concept that the feeding of HER containing diets affects the functioning of the mitochondria have been presented by Clouet et al. (1976). They showed that mitochondria from hearts of rats fed a diet rich in rapeseed oil for two months were more numerous and of greater size as compared with control rats. Biochemical data showed that this increased growth was not accompanied by an increase in enzyme content. They suggested that these mitochondria would oxidize usual fatty acids in suitable conditions, but that this ability would be more reduced in drastic conditions. Dow-Walsh et al. (1975) also showed that mitochondria from rapeseed oil fed rats were more dependent on the presence of heparin for protection against in vitro

loss of oxidative function than mitochondria from control animals and suggested that the fatty acid changes in membrane phospholipids caused by HER feeding could result in increased fragility of mitochondrial membranes in vitro.

These observations suggest that a possible explanation for the increase in liver size is that if liver function is being hampered in some way by rapeseed oil feeding, the liver may enlarge to compensate for this decreased functional ability. Liver function was not tested in these experiments and thus it cannot be stated for certain that this is a factor, but it is a possibility and is open for future study.

SUMMARY

1. Chicks fed diets containing 20 parts HER from 4-28 days of age consumed less energy and grew at a slower rate than chicks fed comparable diets containing either LER or SFO. Growth and energy consumption of chicks fed diets containing LER was intermediate between and significantly different from that of chicks fed either the HER or SFO containing diets.
2. Studies showed that chicks fed 20 parts HER from 4-28 days of age had a significantly greater liver to body weight ratio than chicks fed 20 parts LER, which in turn, had a significantly greater liver to body weight ratio than chicks fed 20 parts SFO. The level of fat in livers of chicks fed 20 parts HER was significantly higher than the SFO fed controls, however, the level of fat in livers of chicks fed 20 parts LER was intermediate between that of chicks fed diets containing SFO and HER but was not significantly different from either.
3. Type of oil fed was found to have no effect on level of liver protein, hydroxyproline content of liver protein or phospholipid content of liver lipid when chicks were fed ad libitum from 4 to 28 days of age. The finding that hydroxyproline content of liver protein was similar, irrespective of type of oil fed,

indicates that the increase in weight of livers of chicks fed diets containing HER and LER was not due to proliferation of connective tissues.

4. Irrespective of whether diets containing 20 parts of HER, LER or SFO were fed, liver neutral lipids were found to be more saturated than carcass lipid.
5. Results showed that when diets containing 20 parts of HER were fed liver neutral lipids contained markedly lower levels of erucic and eicosenoic acids than the oil fed and slightly but significantly lower levels than found in carcass lipid. The ability of the liver to modify the fatty acid composition of ingested fat prior to entering the general circulation may help to protect other tissues in the chick from the adverse effects of erucic and eicosenoic acids.
6. Studies showed that liver phospholipids of chicks fed diets containing HER and LER had significantly lower levels of stearic, linoleic and arachidonic acids and higher levels of N-9 fatty acids than when the SFO-containing diets were fed.
7. The liver phospholipids of chicks fed diets containing 20 parts HER were shown to differ from the phospholipids of chicks fed diets containing LER in that they contained erucic acid and also had significantly higher levels of eicosenoic and lower levels of linoleic and arachidonic acids.

8. Whether the aforementioned changes in fatty acid composition of phospholipids contributed to structural-functional changes in the liver and to the observed increase in liver size when diets containing HER and LER rather than SFO were fed is unknown.

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APPENDIX

Determination of Hydroxyproline

The Neuman and Logan (1950) technique for the determination of hydroxyproline in protein hydrolyzates consists of:

1) oxidation of hydroxyproline with hydrogen peroxide in the presence of alkaline copper sulphate.
2) destruction of excess peroxide by heat.
3) reaction of the oxidation product with p-dimethyl-aminobenzaldehyde by heating in the presence of dilute sulphuric acid to produce a red color, the intensity of which is compared with a standard.

In the modification by Leach (1960b), stages (1) and (2) of the original method are combined. The oxidation is carried out at 40 C and is completed before the total destruction of the excess peroxide has occurred.

Neuman and Logan prepared their protein hydrolyzates by autoclaving 50 mg of protein with 1.0 ml of 6 N HCl in sealed tubes at 15 pounds pressure (121 C). The hydrolyzates were brought to volume and filtered if necessary. In this experiment the protein hydrolyzate was prepared by autoclaving a 200 mg sample with 5.0 ml of 2 N HCl in sealed tubes at 121 C for nine hours. After hydrolysis the samples were brought up to volume (5.0 ml) and filtered.

The reagents used were the same as those employed by Leach (1960):

0.05 M copper sulphate in water
2.5 N (approx.) sodium hydroxide
6% (approx.) hydrogen peroxide
3 N (approx.) sulphuric acid
5% p-dimethylaminobenzaldehyde

Standard L-hydroxyproline solution were prepared by dissolving 0.05 g hydroxyproline in about 400 ml of water. Twenty milliliters of concentrated HCl were added to prevent microbiological destruction and the solution was then made up to 400 ml with water. The 100ug/ml solution was diluted to give concentrations of 5, 10 and 15 ug of hydroxyproline/ml.

The solution under test was to contain between 5 and 15 ug of hydroxyproline/ml, thus one milliliter of hydrolyzate was diluted to 100 ml with distilled water. An aliquot containing between 5 to 15 ug hydroxyproline was placed into a 1 inch by 6 inch test tube, and one ml of 0.05 M copper sulphate and one ml of 2.5 N sodium hydroxide were added. This was mixed by gently swirling and placed in a water bath at 40 C. When the contents reached 40 C (5 minutes), one ml of 6% hydrogen peroxide was added and the contents mixed by swirling. The tubes were left in the bath for 10 minutes, but were occasionally removed and the contents swirled. The tubes were cooled with tap water, then 4 ml of 3 N sulphuric acid and 2 ml of 5% p-dimethylaminobenzaldehyde were added, the contents of the tubes being swirled after each addition.

The tubes were then placed in a water bath at 70 C for 16 minutes. The optical density was determined at 560 mu using a spectrophotometer. Each test solution was measured against a blank solution and then compared with the standards to obtain the amount of hydroxyproline in the sample. This was then expressed as a percentage of the protein content.

Determination of liver lipid and phospholipid

Liver lipid was determined by extraction of a portion of the freeze dried liver for sixteen hours with a mixture of chloroform and methanol (2:1 v/v) in a Goldfish apparatus (Feigenbaum and Fisher, 1963). The solvent was removed by heating the samples on a steam bath while a stream of nitrogen was directed into the flasks. The samples were then re-extracted with petroleum ether, transferred quantitatively into test tubes and centrifuged at 5000 rpm for 10 minutes. The supernatant was decanted into weighed test tubes and the process was repeated, washing the precipitate twice with petroleum ether and decanting the supernatant into the weighed test tubes. The solvent was removed under a stream of nitrogen. The test tubes were dried at 100 C for 10 minutes, cooled in a dessicator and weighed. The weight of the material was considered to be liver lipid.

After the total lipid content was determined the phospholipids were separated from neutral lipids using a modification of the method of Hanahan et al. (1957). One ml of petroleum ether and 10 ml of cold acetone were added to each sample and then the stoppered test tubes were placed in the freezer at -25 C overnight to allow complete separation of the phospholipids. The next day the samples were centrifuged at 5000 rpm for 10 minutes and the supernatant was transferred into another set of

weighed tubes. The phospholipid was washed twice with cold acetone and the washings were added to the weighed tubes. Traces of acetone were removed from the phospholipid samples under a stream of nitrogen gas, then the samples were dried at 100 C for 10 minutes, cooled in a dessicator and weighed. The weight of the material was considered to be liver phospholipid. The sample was then dissolved in petroleum ether and stored at -25 C until analyzed for fatty acid composition.